

GB toxicity reassessed using newer techniques for estimation of human toxicity from animal inhalation toxicity data: New method for estimating acute human toxicity (GB)

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ABSTRACT: Estimated human inhalation toxicity values for Sarin (GB) were calculated using a new two independent (concentration, exposure time), one dependent (toxic response), non-linear dose response (toxicity) model combined with re-evaluated allometric equations relating to animal and human respiration. Historical animal studies of GB toxicity containing both exposure and fractional animal response data were used to test the new process. The final data set contained 6621 animals, 762 groups, 37 studies and 7 species. The toxicity of GB for each species was empirically related to exposure concentration (C ; mg m^{-3}) and exposure time (T ; min) through the surface function $Y = b_0 + b_1 \text{Log}_{10}C + b_2 \text{Log}_{10}T$ or $Y = b_0 + b_2 \text{Log}_{10}CT$ where Y is the Normit, b_0 , b_1 and b_2 are constants and n is the 'toxic load exponent' (Normit is $\text{PROBIT} - 5$). Between exposure times of 0.17 and 30 min, the average value for n in seven species was 1.35 ± 0.15 . The near parallel toxic load equations for each species and the linear relationship between minute volume/body weight ratio and the inhalation toxicity (LC_{50}) for GB were used to create a pseudo-human data set and then an exposure time/toxicity surface for the human. The calculated n for the human was 1.40. The pseudo-human data had much more variability at low exposure times. Raising the lower exposure limit to 1 min, did not change the LC_{50} but did result in lower variability. Raising the lower value to 2 min was counterproductive. Based on the toxic load model for 1–30 min exposures, the human GB toxicities (LC_{01} , LC_{05} , LC_{10} and LC_{50}) for 70 kg humans breathing 15 l min^{-1} were estimated to be 11, 16, 36 and 83; 18, 25, 57 and 132 and 24, 34, 79 and 182 $\text{mg} \cdot \text{min m}^{-3}$ for 2, 10 and 30 min exposures, respectively. These values are recommended for general use for the total human population. The empirical relationships employed in the calculations may not be valid for exposure times >30 min. Copyright © 2005 Crown in the right of Canada. Published by John Wiley & Sons, Ltd.

KEY WORDS: GB; nerve agents; toxicity; humans; animals; new methods

Introduction

In the post World War II period, the toxicity of Sarin (GB) was studied extensively as the Western Nations mounted international cooperative programs to assess the military impact of the then newly appreciated organophosphate nerve agents and to develop protective and therapeutic measures to counteract the observed threat. These assessments required estimates of the toxicity of the agents for humans, particularly LC_{50} or better for protection. The most reliable source of human toxicity data is fully documented human exposures. However, such information is difficult to obtain and what little data

that are available contain only marginal estimates of the dosages involved.

In the absence of human data, the toxicologist must rely upon animal toxicity data and make extrapolations to the human condition. Experimental/mathematical procedures are available for the estimation of human LC_{50} values (Guyton, 1947; Crostall and Widdicomb, 1961; Krasovskii, 1976; Blackburn, 1984; Phalen, 1984; Gross, 1987; Salem, 1987; Snipes, 1988; Anon, 1994; McClellan, 1995; Koller and Henderson, 1997; Grotte and Yang, 1998; Harkema, 1999). However, these procedures fail when applied to LC_{01} and LC_{10} and estimations of these values have had to rely upon subjective comparisons and multiple assumptions that vary between experts with the result that much controversy surrounds this activity. Consequently, toxicologists have been very reticent about providing estimates of LC_{01} and LC_{10} .

Currently, the most common method used to define the median or 50% toxicity of a substance (LD_{50}) is to generate, experimentally, a sigmoid toxicity exposure-response curve at a fixed exposure time. The mortality—

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exposure data are plotted as a linear PROBIT relationship of per cent effect vs dosage (PROBIT = Normit + 5. This transformation was originally done because mechanical calculators could not handle negative numbers. Normits are used in this study). Some standard techniques have been developed (Bliss, 1938a,b; Litchfield and Wilcoxon, 1949; Anon, 1987; Tallarida and Murray, 1987) for estimating the LD_{50} . In inhalation toxicity studies, the dose, which usually cannot be measured, is substituted by the exposure concentration (C)–time (T) product or CT and the measure of toxicity is the LCt_{50} or the concentration of substance which, when inhaled for a fixed time, will produce 50% mortality in a given population. Unfortunately, with nerve agents, as exposure times change the LCt_{50} does not remain constant (Gates and Renshaw, 1946; McPhail and Barrett, 1949; Trurnit *et al.*, 1953; Koller and Henderson, 1997; Grotte and Yang, 1998), i.e. Haber's rule (Haber, 1924) of $CT = k$ is not obeyed. In fact, in many relationships (Phalen, 1984; ten Berge *et al.*, 1986; Gelzleichter *et al.*, 1992; Fairhurst and Turner, 1993), for a fixed percent effect the toxicity correlates to the exposure concentration and time by the equation

$$k = C^n T \quad (1)$$

where k is a numerical constant, C is the exposure concentration of toxic material (mg m^{-3} in this paper), T is the exposure time (min in this paper) and n is the 'toxic load exponent'. The n value can range from 0.85 to >3.5 (ten Berge *et al.*, 1986). It cannot be emphasized enough, that this $C^n T$ model is an empirical relationship that must be derived from experimental values and is therefore limited by the experimental data available. The values derived are only as good as the fit of the model to the data. Further, the validity of the estimates will vary with the dispersion and reliability of the data used in the calculations, i.e. the better the fit to the model, the better the data set and the better the resulting toxicity estimates. There are known instances in which the model does not apply (Phalen, 1984; Gelzleichter *et al.*, 1992).

The common method employed (Guyton, 1947; Phalen, 1984; Snipes, 1988; McClellan, 1995) for evaluating the constant n (Eqn. (1)) is to do a series of experiments to obtain LCt_{50} values at different fixed exposure times (i.e. a Normit plot with one independent and one dependent variable (1V) as Eqn. (2)) and then to plot (Fig. 1) the resulting $\log_{10}(LCt_{50})$ values obtained against $\log_{10}(T)$. The slope of the resulting line is $(1-1/n)$. These processes require an experimental design (progressive exposure concentrations at fixed times) which was not always followed by early workers studying the toxicity of GB. However, this does not mean that the data gathered are of no consequence.

The process described above generally provides reasonable estimates of the effect of exposure time upon the

LCt_{50} . However, attempts to obtain other values (such as LCt_{10}) by the same method usually fail because of the high variability or dispersion in the slope and intercept of the Normit line encountered in the experimental data from successive 1V experiments. This effect is clearly seen when slopes of the Normit lines are plotted against exposure time (Fig. 1). In turn, the toxicity values that are removed from the central LCt_{50} , also will be progressively scattered.

The procedure above for the estimation of the effect of exposure time, produces a linear relationship between the toxicity (LCt_{50}) and exposure time but, in reality, what is being generated is the 50% line of a surface — a two variate (2V) surface plot relating toxicity (LCt_{50}), C and T . The usual 1V relationship at a fixed exposure time is a slice through the 2V surface at a given value of T . The other relationship obtained when exposure time is varied at fixed C (a procedure often used in radiation studies) is a slice through the 2V surface at 90° to that generated at fixed T . Using modern computer techniques, it is practical to go directly to a 2V planar surface (model) relating exposure concentration, exposure time and percent effect (ten Berge *et al.*, 1986; Yee, 1996a,b, 1999; Yee and Ye, 1996; Yee *et al.*, 1998). In the 1V method, the relationship at a fixed exposure time T is expressed by

$$Y = a_0 + b_1 \log_{10} C \quad (2)$$

This equation can be generalized to the 2V relationship, also referred to as the 'toxic load model'

$$Y = b_0 + b_2 \log_{10} C^n T \quad (3)$$

$$\text{or } Y = b_0 + b_1 \log_{10} C + b_2 \log_{10} T \quad (4)$$

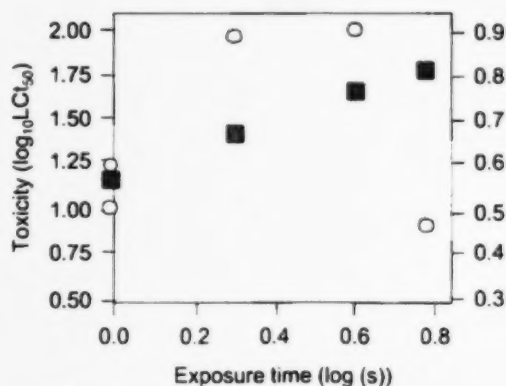


Figure 1. LCt_{50} and Normit slope values are seldom both linear as exposure time changes. The toxicity (LCt_{50}) of GB to rats (■) varies as the exposure time increases in a log-linear fashion. The slope of the Normit lines (○) used to generate the LCt_{50} do not. Data plotted are from McPhail, 1955

involving the exposure time, T , explicitly. Here, Y is the Normit value and a_0 , b_0 , b_1 and b_2 are empirical numeric constants. In Eqn. (4), $n = b_1/b_2$. Note that constants a_0 and b_0 are dependent upon the units of C and T whereas the other constants and n are not. With sufficient data available, the empirical constants (and, hence, the 2V planar surface) can be evaluated easily using a modern desktop computer and a maximum likelihood method (Yee, 1996b; Yee *et al.*, 1998). From the constants of the 2V surface, estimates are readily obtained for the values of LC_{01} , LC_{05} , LC_{50} and LC_{95} as a function of exposure time.

Because the 2V calculations encompass all CT values, a fixed exposure time is not imposed upon the experimental design and many early experiments, carried out using variable exposure times and/or concentrations, may be used with the 2V model. Further, as the first step in the 2V calculation is to reduce all the data to dead : alive (1 : 0 or 0 : 1) type individual data, data collected by the 'up and down' method (Dixon and Mood, 1948; Brownlee *et al.*, 1953; Bruce, 1985) commonly used with large animals may be incorporated and/or evaluated directly. Thus, the amount of data available for evaluation may be greatly increased.

Having achieved reasonable methods for estimation of the toxicity to animals, the second part of the problem, and the most controversial, is the estimation of human toxicity from the animal data. This process requires some form of correction for body size. In general, the inter-species relationships between body size/weight/shape, physiology and toxicology have been well defined (Stahl, 1967; Stahl and Gummerson, 1967; Stauffer, 1975; Krasovskii, 1976; McMahon and Bonner, 1983; Calder, 1984; Schmidt-Neilsen, 1984; Bennett and Harvey, 1987; Bide *et al.*, 1997, 2000; West *et al.*, 1997). Leading from this, various relationships have been developed for estimation of human toxicity from animal data (Guyton, 1947; Croxhill and Widdicomb, 1961; Krasovskii, 1976; Blackburn, 1984; Bennett and Harvey, 1987; Homs and Crosier, 1987; Snipes, 1988; Harvey and Pagel, 1991; Anon., 1994, 1998). For GB, an excellent correlation is obtained between the minute volume to body weight ratio and the observed toxicity (Koller and Henderson, 1997; Bide *et al.*, 1998a,b, 1999, 2001, 2004; Grote and Yang, 1998). This relationship, used in conjunction with the allometric relationship between body weight and respiratory volume for animals and man (Phalen, 1984; Snipes, 1988; Bide *et al.*, 1997, 2000), forms the basis for the method proposed in this study for obtaining human toxicity estimates at short exposure times.

The objective of this study was to describe a process to obtain reasonable estimates of human inhalation toxicity developed from, and fully based upon, animal toxicity data without resorting to controversial 'selection' processes. The values obtained were to include LC_{01} ,

LC_{05} and LC_{50} for a range of exposure times and the limits of the information were to be clearly defined. This paper describes a process, using the newer computational methods now available on desktop computers, first, to obtain 2V representations of the animal toxicity data for several species and then an estimated 2V surface for the human from which specific human toxicity estimates may be derived. The wealth of animal toxicity data available for GB has been used to illustrate the process.

This work has been presented at several meetings (Bide *et al.*, 1998a,b, 1999, 2001) to encourage discussion and criticism. To date, the responses have been positive.

Methods

Data concerning the toxicity of GB (isopropyl methylphosphonofluoridate, Sarin, CAS 107-44-8, MW 140.1) were gathered from the open and the military literature. Since most of the work was reported before the advent of electronic literature databases, a manual search of Chemical Abstracts was undertaken for the years 1945–1965 followed by searches for references to the papers identified.

From the individual animal response data, the LC_{01} , Normit slope and intercept were calculated by one or both of the classic methods (Bliss, 1938a,b; Litchfield and Wilcoxon, 1949; Anon., 1987; Tallarida and Murray, 1987). In some instances, when the classic methods could not be employed because of insufficient data in the critical 16%–84% toxicity range, a 1V version (a special case of the 2V version) of the DRDC Suffield program was also used. The 2V surface calculations were carried out using programs prepared at DRDC Suffield (Yee, 1996b; Yee *et al.*, 1998; Bide *et al.*, 2004) in S-plus, Version 3.4 for UNIX (Mathsoft, Inc., Seattle, Washington) running on a Hewlett-Packard model 715/50 workstation under HP-UX (Hewlett-Packard, 3000 Hanover St., Palo Alto, California). All the computations were performed in double precision to minimize the effects of truncation on the statistical calculations.

A step-by-step procedure to calculate the human toxicity estimates is provided. Briefly stated, the process assembles and analyses the data for each species, calculates the toxicity, concentration, exposure time surface for each species, establishes an interspecies relationship, creates a pseudo-human data set from the animal data and uses the pseudo-human data to calculate a 2V surface for the human. Specific toxicological estimates may then be read from the surface.

The literature values for the toxicity of GB to humans, both published estimates and reports of human exposures, were reviewed and the values compared with the estimates obtained from the animal studies by the proposed methods.

Evaluation of the Available Animal Toxicity Data

Literature values were collected from as many sources as possible to provide the basic data for this project. For a study to be included in the basic data set, which considered only GB toxicity to mammals, the 'raw' experimental data, i.e. C , T and the percent response, had to be available for re-evaluation. In all, studies involving nine species were identified. Following the procedures outlined below, some of the data were eliminated from the final database. A summary of the animal numbers, groups, studies, high and low exposure times and some representative toxicity values are provided in Table 1.

The object, for each species, was to create a summary data set that was as large as possible while being cohesive and representative. To this end, a simple process of inter-laboratory quality assurance was applied. The data from each paper were recalculated to provide concentration in mg m^{-3} and time in min, the data were re-analysed and compared with the authors' stated result and, when possible, a 2V surface calculation was made. All the data for that species were then combined, a 2V surface calculated and, at one T , the ratio of $\text{LC}_{50}/\text{LC}_{10}$ (Ratio) was calculated. This Ratio was used as a simple, but effective, measure of the dispersion of the data. It is directly related to the 1V Normit slope obtained at fixed T ; (Eqn. (2)). Also, because the 2V surface (toxic load model) is a planar surface, the Ratio is constant for all T . For each experiment, the CT values for each group of animals were plotted on a graph of $\log_{10}CT$ vs $\log_{10}T$ and lines representing the LC_{10} , LC_{50} and LC_{90} from the combined 2V surface were added to each graph. A similar graph of the combined data was also prepared. Examination of these graphs should indicate whether the data from one (or more) experiment(s) were clearly separated from the

remainder (Fig. 2). If there were experiments with data separated from the rest, those data were removed, experiment by experiment, and, each time, the 2V toxicity surface for the species was recalculated and re-plotted. If the result, in each case, was a marked reduction in the Ratio and a marked increase in the slope constants, b_1 and b_2 , the data from that experiment were considered for exclusion from the final data set. This process was repeated until a data set was obtained for which most of the remaining data were within the LC_{10} and LC_{90} lines of the final 2V surface. The data were then re-examined and a final decision was made concerning the content of the data set for that species. Representative toxicity values for each species were extracted (Table 1).

Exclusion of Data for <10 s Exposures

As a final step in the process, the animal data were arbitrarily truncated to eliminate all exposures of less than 10 s. In mice, rats, guinea pigs and monkeys, the toxicity resulting from exposures <10 s appeared to follow a different pattern from that of longer exposures. There were no clear indications of a cause for this phenomenon although the extreme technical difficulty of obtaining a reliable exposure of <10 s may be indicated. In several of the species used (cat, dog, monkey) for which the time for a single breath is more than 1 s (Altman and Dittmer, 1956; Gautier, 1984; Karel and Weston, 1946; Mauderly, 1974), it becomes almost impossible to ensure that the animal was inhaling during the exposure and T becomes meaningless. For rabbits, there were no data for exposures <1 min. For cats and dogs, the exclusion of data for exposures <10 s made no difference to the resulting toxicity calculations because all animals exposed for <10 s survived and the recorded CT values were well separated

Table 1. Statistics of the data base: Numbers of studies, groups, animals and selected toxicities

Species	Total studies	Accepted studies	Numbers in accepted studies		Exposure times (min)		LC_{50} ($\text{mg}\cdot\text{min m}^{-3}$)	
			Groups	Animals	Low	High	2 min	10 min
Mouse	10	9	229	3350	0.17	30	215	316
Rat	15	10	254	2460	0.17	30	114	192
G. pig	5	3	61	372	0.17	12	87	145
Rabbit	5	4	59	136	0.17	15	78	122
Cat	3	3	48	82	0.17	10	62	90
Dog	6	5	54	91	0.25	10	88	122
Monkey	5	3	67	130	0.17	10	45	67
Sheep	1	0					219	227
Pig	1	0						35
Total	51	47	762	6621	0.17	30		
Pseudo-data sets								
Dog		32	708	6530	0.17	30	90	142
Human		17	762	6621	0.17	30	27	47

Data not used in the calculations.

* 70 kg human breathing 15 l/min.

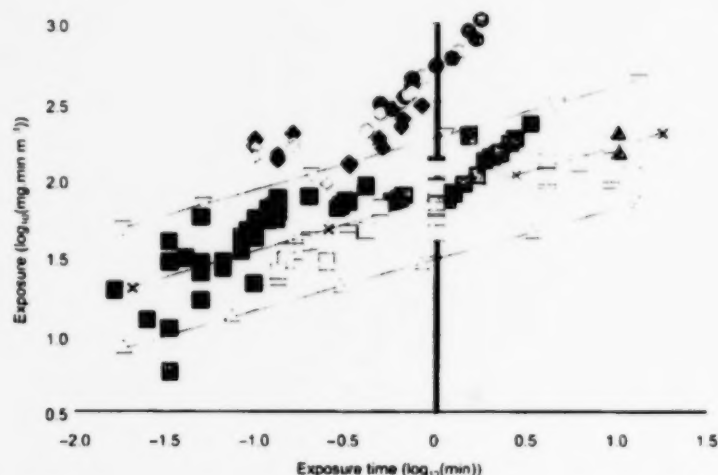


Figure 2. The total guinea pig data with the LC_{05} , LC_{50} and LC_{95} lines superimposed. Each point represents the position (CT) of a group in that data set. Two of the 5 studies depicted were discarded from the final data set. As only single animals were exposed, the results were alive ($\square$, $\triangle$) or dead ($\bullet$, $\blacklozenge$) animals. Each group of animals in the other three studies are represented at the exposure CT without any attempt to represent toxic response. The lines ($\times$, Δ , ∇) represent the LC_{50} , LC_{05} and LC_{95} , respectively, of the 2V surface calculated from the data of the 3 accepted studies

from values which were predicted to be lethal. For exposures >10 s, the data for all seven species appeared to follow a similar pattern of toxicity change under a power law relationship as the exposure time increased, i.e. the 2V surface or toxic load model (Eqn. (3)).

Toxicity in Guinea pigs

The guinea pig data are presented first to illustrate the process of evaluating the animal data.

The literature search provided five papers (Rotariu *et al.*, 1945; McPhail and Barrett, 1949; Trumit *et al.*, 1953; Cullumbine *et al.*, 1955; Koon *et al.*, 1960) with animal response data for guinea pigs exposed to GB. Using all of the guinea pig data, the calculated 2V surface was very shallow as indicated by the low values of b_1 and b_2 and the very large value of the Ratio (Table 2). When the data were plotted (Fig. 2), the data from one study

(Koon *et al.*, 1960) were clearly aberrant and the data from a second study (Cullumbine *et al.*, 1955) also appeared to be higher than the rest. When data from the first study (Koon *et al.*, 1960) were removed and the surface recalculated, significant improvements were achieved in the Ratio, b_1 and b_2 (Table 2). Removal of the second study (Cullumbine *et al.*, 1955) improved the values again and when the <10 s data were removed, there was another marked change in the Ratio and in the constants of the surface. Removing other experimental data also produced changes in the surface constants but these were much smaller and not considered great enough to warrant the further reduction in the numbers of animals in the data set.

Toxicity in Mice

The literature search provided ten studies in nine reports (Rotariu *et al.*, 1945; McGrath and Fuhr, 1948

Table 2. Surface constants for guinea pig data

Item	Treatment	Calculated surface constants				
		b_0	b_1	b_2	n	Ratio
[1]	All data	-2.0492	1.1185	1.0296	1.0656	873
[2]	[1] less Koon <i>et al.</i> , 1960	-4.5300	2.0120	1.6738	1.2021	43
[3]	[2] less Cullumbine <i>et al.</i> , 1955	-7.4042	2.8936	2.3728	1.2195	13.7
[4]	[3] ≥ 10 s	-7.8797	4.2902	2.8510	1.5048	5.84

Bold print indicates the accepted data surface

Table 3. Surface constants for mouse data

Item	Treatment	Calculated surface constants				
		b_0	b_1	b_2	n	Ratio
[1]	All data	6.2700	2.6383	2.2517	1.1717	7.7
[2]	[1] less high Barrett, 1951	-6.3479	2.7736	2.1582	1.2847	15.2
[3]	[2] ≥ 10 s	-8.3461	3.6907	2.7947	1.3206	7.79

Barrett, 1951, contains two studies with different results. The author questioned the data set with higher values. Only the low data set was used. Bold print indicates the accepted data surface.

Table 4. Surface constants for combinations of rat data

Item	Treatment	Calculated surface constants				
		b_0	b_1	b_2	n	Ratio
[1]	All rats	5.1513	1.5108	1.1266	1.3411	180
[2]	[1] less Barrett, 1951	6.4289	2.0198	1.3475	1.4989	42.5
[3]	[2] less Cullumbine <i>et al.</i> , 1955	7.5020	2.4069	1.5897	1.5140	23.2
[4]	[3] less Cresthull <i>et al.</i> , 1953	7.8613	2.4971	1.6450	1.5180	20.7
[5]	[4] less Callaway and Blackburn, 1954	-8.8637	2.8208	1.8703	1.5082	14.7
[6]	[5] less McPhail, 1955 ≥ 10 s	-6.20900	3.5048	2.3603	1.4849	8.68

McPhail, 1955 was eliminated for item [6] because all exposures were < 10 s. Bold print indicates the accepted data surface.

unpublished report; Muir and Callaway, 1948a; Dixon and Koon, 1949; McPhail and Barrett, 1949; Barrett, 1951; Cresthull *et al.*, 1953, 1951; Trumit *et al.*, 1953) with animal response data for mice exposed to GB. In addition, some recent 20 min exposure data from DRDC Suffield were included (Bide and Risk, 2002, 2004). Of the ten studies (Table 3), the data from two (Barrett, 1951; Cresthull *et al.*, 1951) appeared to be separated from the body of the rest. When the data from Cresthull *et al.*, 1951 were removed, the Ratio increased and the slope constants b_1 and b_2 decreased so these data were retained. The second report (Barrett, 1951) contained two studies with mice in which a different exposure apparatus was used with different results. Based on the author's comments, only the lower values were included in the data base. When the data for exposure < 10 s were removed, marked improvements in the Ratio, slope constants and n were achieved.

Toxicity in Rats

The literature search provided 12 reports (Rotariu *et al.*, 1945; McGrath and Fuhr, 1948, unpublished report; Muir and Callaway, 1948b; Fish, 1949; McPhail and Barrett, 1949; Barrett, 1951; Cresthull *et al.*, 1953; Parker and McPhail, 1953; Trumit *et al.*, 1953; Callaway and Blackburn, 1954; Cullumbine *et al.*, 1955; McPhail, 1955) with animal response data for rats exposed to GB. In addition, recent data for exposures 20 and 30 min

(Mioduszewski *et al.*, 2000, 2001¹) and some data from the DRDC Suffield archives (McPhail, unpublished data, 1950, 1951) were included. When the data for the two studies furthest removed from the remainder (Barrett, 1951; Cullumbine *et al.*, 1955) were progressively taken out of the calculation (Table 4), improvements in both the Ratio and the slope constants b_1 and b_2 were achieved. The removal of two more studies (Cresthull *et al.*, 1953; Callaway and Blackburn, 1954) again improved the situation. When the data for exposures < 10 s were removed, a further marked improvement was achieved. At this point in the data analysis, one study (McPhail, 1955) was lost because all exposures were < 10 s. Removing more data did not have useful effects.

Toxicity in Rabbits

The literature search provided five studies (Rotariu *et al.*, 1945; McGrath and Fuhr, 1948, unpublished report; Silver *et al.*, 1950, 1951 unpublished reports; Ainsworth, 1954) with animal response data for rabbits exposed to GB. In one paper (Ainsworth, 1954), exposure data were reported for six rabbits of which four received exposures 2–20 times higher than lethal. In addition, the exposure times were not clearly established so this study was not

¹These data were obtained originally in 1998 as a personal communication. Data for shorter and longer exposure times, included in the published papers, were not included.

used. The other rabbit data were accepted. There were no data for <10 s exposures. The calculated constants for the exposure-response surface were: $b_0 = -14.1908$, $b_1 = 7.8466$, $b_2 = 5.6377$, $n = 1.3918$ and Ratio = 2.63.

Toxicity in Cats

The literature provided three studies (Rotariu *et al.*, 1945; McGrath and Oberst, 1952; Trurnit *et al.*, 1953) with usable mortality data. Two of these (Rotariu *et al.*, 1945; McGrath and Oberst, 1952) contained 10 min exposures only. In the third study (Trurnit *et al.*, 1953), cats were exposed to GB for times between 1.2 and 240 s. Because the toxicity data were collected with both variable C and T values, the data were analysed by the 2V procedure. The 10 min LC₅₀ values were judged to be comparable within experimental error. Scrutiny of the data for the shortest exposure times showed that no lethal response was elicited and these data had no effect upon the resulting 2V surface calculations.

All the data from the three papers were deemed acceptable. For consistency with the data from other species, the exposures of <10 s were removed. A 2V surface, calculated from this data set had the constants: $b_0 = -18.5512$, $b_1 = 10.7994$, $b_2 = 8.1973$, $n = 1.3174$ and the Ratio = 2.02.

Toxicity in Dogs

The literature provided six studies (Rotariu *et al.*, 1945; McGrath *et al.*, 1952; Trurnit *et al.*, 1953; Punte *et al.*, 1954; Smith, 1954; Koon *et al.*, 1958) with animal re-

sponse data for dogs exposed to GB. A calculation using all of the dog data produced a shallow, broad, 2V surface with low slope constants and a large Ratio (Table 5). In one study (Koon *et al.*, 1958), the sizes of the dogs were very disparate, much of the data were above the range of the remaining dog data and the variability of the data was very large. Removing the data from this study from the surface calculations markedly reduced the Ratio and increased the slope constants b_1 and b_2 . When exposures of <10 s were removed from the dog data set, there was a further decrease in the Ratio and increases in the b_1 and b_2 constants. The marked reduction in the n value as the <10 s data were removed is the result of the combination of the small numbers of animals involved (91) and the majority of the animals (69) being exposed at either 1 or 2 min, values central to the majority of the animal data. Thus, the n value of 1.1057 did affect (lower) the average n for the animal species but did not appear to affect the subsequent calculation of pseudo-human values because the range of T values was small and central to the pseudo-human distribution.

Toxicity in Monkeys

The literature provided five studies (Rotariu *et al.*, 1945; Muir *et al.*, 1952; Trurnit *et al.*, 1953; Cullumbine *et al.*, 1955; Cresthull *et al.*, 1957) with animal response data for monkeys exposed to GB. Using all of the monkey data, the calculated constants indicated an exposure-response surface that was very broad and shallow (Table 6). The data from two papers (Muir *et al.*, 1952; Cullumbine *et al.*, 1955) contained much higher toxicity values and were progressively removed from the database

Table 5. Surface constants for dog data

Item	Treatment	Calculated surface constants				
		b_0	b_1	b_2	n	Ratio
[1]	All dog data	-3.0102	1.6523	1.8658	1.0554	96
[2]	[1] less Koon <i>et al.</i> , 1958	-30.7796	5.2502	3.6910	1.4249	4.22
[3]	[2] ≥ 10 s	-13.0059	6.7965	6.1468	1.1057	3.05

Bold type indicates the accepted data surface.

Table 6. Surface constants for monkey data

Item	Treatment	Calculated surface constants				
		b_0	b_1	b_2	n	Ratio
[1]	All data	-1.9281	1.0912	0.8547	1.2768	1034
[2]	[1] less Cullumbine <i>et al.</i> , 1955	2.1803	1.2917	0.9246	1.3690	352
[3]	[2] less Parker and McPhail, 1953	2.9350	1.8908	1.4075	1.4461	84
[4]	[3] ≥ 10 s	-4.9943	3.2018	2.4851	1.2864	10.7

Bold print indicates the accepted data surface.

Table 7. Constants of predicted 2V surfaces for all species

Species	b_0	b_1	b_2	n	Ratio
Mouse	8.3261	3.6007	2.7947	1.3206	7.75
Rat	-6.8000	3.6028	2.7603	1.4849	8.68
Guinea pig	-7.8797	4.2002	2.8510	1.5048	8.84
Rabbit	14.1807	7.8466	5.6377	1.3918	2.63
Cat	-18.5512	10.7994	8.1973	1.3174	2.02
Dog	13.0050	6.7965	6.1468	1.1057	3.03
Monkey	4.9945	3.2018	2.4851	1.2884	10.77
Average n value i.e. toxic load exponent				1.3448 $\pm$ 0.1457	
Calculated data sets^a					
Dog	6.7881	3.6345	2.5988	1.3985	8.04
Human (by range of exposure times, min)					
0.17–30	5.3097	3.6297	2.6158	1.3876	8.06
0.33–30	6.3087	4.3214	3.1060	1.3913	5.77
0.5–30	6.2387	4.2998	3.0457	1.4117	5.82
1–30	-6.5748	4.5242	3.2208	1.4046	5.34
2–30	6.9698	4.7807	3.4296	1.3939	4.88

^aValues for the pseudo-dog and pseudo-human data sets generated for this study.
 Bold type indicates recommended surface for general use.

with concomitant improvements in b_1 , b_2 and Ratio. The n value first increased as the above papers were removed and then was lowered when the exposures of <10 s were removed. However, the b_1 , b_2 and Ratio values improved further with the removal of exposures of <10 s.

Toxicity in Sheep

The literature search provided one study (Cullumbine *et al.*, 1955) with animal response data for sheep exposed for 0.17 and 2 min to GB. The two LC_{50} values of 227 and 219 mg.min m⁻¹, respectively, were not consistent with the data for the remaining animal species in that the higher LC_{50} value was recorded at the shorter exposure time. The sheep data were not included in the human estimate calculations.

Toxicity in Pigs

One study (Crook *et al.*, 1952) was found with animal response data for pigs exposed for 10 min to GB. The LC_{50} was 34 mg.min m⁻¹. The pigs used were very young and the authors of the paper judged the data to be non-representative of adults. The pig data were not used in the human estimate calculations.

Toxicity in Birds and Fruit Flies

The toxicities of GB to birds and flies have been reported (McPhail and Barrett, 1949; Cresthull *et al.*, 1953). The avian and insect studies are mentioned for the record only.

Summary of Animal Data

The n values calculated from the data for the seven individual species were generally similar (Table 7). The mean n value was 1.345 $\pm$ 0.146 with a range of 1.1057–1.5048. On a graph, the lines are parallel within experimental error (Fig. 3). The scatter within the data sets, as reflected in the Ratio and the slope constants b_1 and b_2 , is quite wide. The cat and rabbit data, which are the tightest distributions, are from small numbers of both animals and studies (Table 1) and, in the case of the rabbits, from only one laboratory. In contrast, the mouse, rat and monkey data are from many more reports by several laboratories. Some representative toxicity values are provided in Table 1.

Calculation of Human Estimates

Relating Toxicity and Animal Size

In many cases, physiological and toxicological parameters are transposed between animal species using relationships between toxicity and body mass or volume. For inhalation studies, there is an allometric relationship between minute volume and body weight that may be used to define the interactions between species (Guyton, 1947; Crostill and Widdicomb, 1961; Stahl, 1967; Stahl and Gummerson, 1967; Stauffer, 1975; Krasovskii, 1976; McMahon and Bonner, 1983; Blackburn, 1984; Calder, 1984; Phalen, 1984; Bennett and Harvey, 1987; Crosier, 1987; Salem, 1987; Snipes, 1988; Harvey and Pagel, 1991; Anon., 1994, 1998; McClellan, 1995; Bide *et al.*, 1997, 2000; Hons and Koller and Henderson, 1997; West *et al.*, 1997; Grotte and Yang, 1998; Harkema, 1999). For the nerve agent GB, there is an excellent correlation

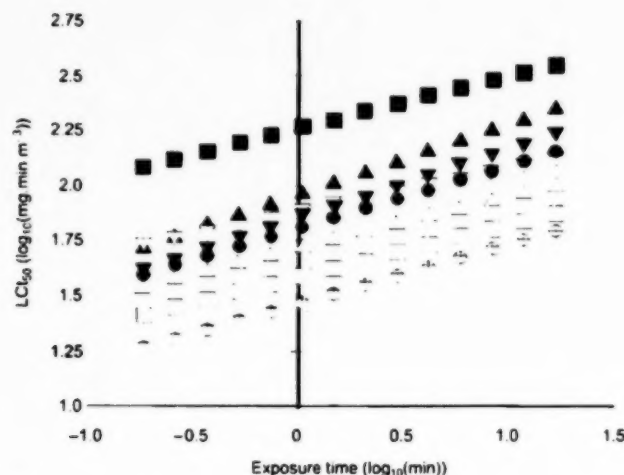


Figure 3. Plots of LC_{50} vs T generated from the 2V surface, calculated for each species, are parallel within experimental error. The plots are: mice (■), rats (▲), guinea pigs (▼), cats (·), dogs (·), rabbits (●), monkeys (·) and, by two calculations, humans (·, +)

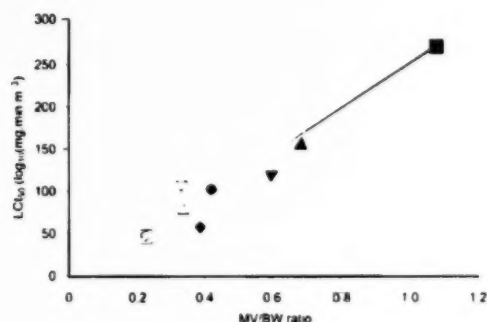


Figure 4. An example of a regression line of MV/BW vs toxicity (LC_{50}). The points are for mice (■), rats (▲), guinea pigs (▼), cats (·), dogs (·), rabbits (●), monkeys (●) and the calculated value for humans (·)

(Fig. 4; Table 8) between toxicity and the minute volume body weight ratio (MV/BW) that may be used to predict toxicity in one species from values in several others (Koller and Henderson, 1997; Bide *et al.*, 1998b, 2001; Grotte and Yang, 1998).

Predicting a Toxicity Surface

The lines of LC_{50} vs exposure time (Fig. 3) may be represented as simple linear equations of the form $y = a + b$ (LC_{50}). If the lines were exactly parallel, the slopes (b) would be equal for all lines and the y intercepts (a) would be the unique feature of each line. To move any line to superimpose upon another would simply require

that a constant be added to the appropriate y intercept. Further, the lines for the species are related via the MV/BW ratios. To obtain the line for another species H , for which the MV/BW is known, a regression of the LC_{50} vs MV/BW is calculated at one T (Fig. 4) for the various species. The toxicity, as $\log_{10}(LC_{50})$, for species H is calculated from the regression line using the MV/BW value for H . To obtain the y intercept for species H , a correction factor is calculated by subtracting the $\log_{10}(LC_{50})$ of H at T from the $\log_{10}(LC_{50})$ at T of species J (for which the LC_{50} vs exposure time line is known) and then subtracting this correction factor from the y intercept of the line for J . Because the lines are parallel, the LC_{50} values for species H may be calculated.

Unfortunately, the LC_{50} vs exposure time lines are not parallel (Fig. 3) and the slopes are not equal but only similar within the limits of experimental error. Therefore, to estimate the LC_{50} vs T relationship for the human (or any other species), a series of regressions of LC_{50} vs MV/BW were made at 14 values of T . All correlation coefficients (r) obtained (Table 8) for the LC_{50} vs exposure time regressions were >0.870 and those for 1–10 min T were >0.91 . Then, using the 'standard' MV/BW value of 0.223 previously established (Bide *et al.*, 1997, 2000) for a 70 kg human, a series of LC_{50} values for the human were calculated from the 14 regression lines. These points, which form a predicted LC_{50} vs T line for the human, were plotted on the same graph as the animal lines (Fig. 3). As noted in the paragraph above, any of the lines for other species may be moved on top of the proposed human line by calculating a correction factor to add to the y -intercept of the line to be moved. The fit

Table 8. Toxicity values for inter-species regression

Species	MV	Exposure times (min)				
	BW	0.53	1.05	2.10	5.3	10.5
Mouse	1.076	156	185	218	273	323
Rat	0.681	75	92	118	150	190
Guinea pig	0.594	44	70	88	110	151
Rabbit	0.416	52	65	79	103	125
Monkey	0.382	31	37	42	53	61
Cat	0.335	48	53	62	78	92
Dog	0.328	77	82	88	96	103
Human	0.223	28	31	35	42	48
Correlation coefficient (<i>r</i>)		0.870	0.900	0.938	0.964	0.973

Values are inhalation toxicity in mg.min/m³

Table 9. Constants for calculating pseudo-dog and -human values

Species	Dog constants	Human constants
Mouse	0.3694	0.7932
Rat	-0.1093	0.5147
Guinea pig	0.0297	0.3652
Rabbit	0.0685	0.3461
Cat	-0.1745	0.2334
Dog	0.0	-0.4069
Monkey	0.3350	-0.0859

obtained will depend upon how far from parallel to two lines really are. If a correction factor is calculated near the middle of the two lines, the values will be close in the middle and more separated at the ends. The average values of the constants to be added to each animal line to move it to the human position were calculated in this fashion (Table 9).

As the LC_{50} vs exposure time lines may be moved, so can all of the data from which the lines were generated. The same LC_{50} vs T line for species H is obtained whether the toxicity vs exposure time line for J is calculated and then the line moved or the raw data for J are moved and then the LC_{50} vs T line for H is calculated. In the latter case, a 2V surface is created instead of just the LC_{50} line. Thus, to obtain a predicted or 'pseudo'-population for one species from the data of another, an adjustment constant is added to the $\log_{10}(CT)$ of each data point to move the entire data distribution. A surface calculation may then be performed on the 'pseudo' data set that has been created and toxicity estimates may be obtained from the surface. Because the 2V surface encompasses all CT values, estimates of LC_{10} , LC_{50} and LC_{90} are readily obtained. The estimation of toxicity may be enhanced by moving all of the available animal data, creating a much larger 'pseudo-species' database for the 2V calculation.

Validation of the Predictive Process

As a verification of this 'pseudo-species' approach, a 'pseudo-dog' data set was created. The dog data were chosen to represent the worst case from among the species available. The appropriate constants (Table 9) were calculated as described above and added to each $\log_{10}(CT)$ of the other species to create a pseudo-dog data set for 0.17–30 min exposures. A 2V toxicity surface for the pseudo-dog was then calculated. The real- and pseudo-dog 2V surfaces were compared by plotting the LC_{10} , LC_{50} and LC_{90} lines for both surfaces upon a scatter diagram of the real dog data (Fig. 5). The surface constants for the pseudo-dog data may be compared with those for the real dog data (Table 7). As expected, the Ratio and b_1 and b_2 constants indicate a shallower, flatter surface for the pseudo-dog population. Despite this, the LC_{10} and LC_{90} lines (Fig. 5) of the pseudo-dog surface enclose the majority of the dog data and the LC_{50} line of the pseudo-dog data is close to that of the real dog data. In fact, either surface may be used to describe the toxicity of GB for the 'standard' dog with a body weight of 10 kg (Blackburn, 1984; Bide *et al.*, 1997, 2000). Calculations for dogs of different size/breed must be done using an appropriate MV/BW ratio obtained from the allometric equations (Bide *et al.*, 1997, 2000) relating respiration and body weight.

Values for Man Estimated from the Animal Data

Using this process described above, a 'pseudo'-human data set was created from all of the accepted animal data for 0.17–30 min exposures and a scatter diagram was prepared (Fig. 6). From these data, a 2V surface was calculated. LC_{10} , LC_{50} and LC_{90} lines were generated and the lines were added to Fig. 6. The LC_{50} line, also added to Fig. 3, was essentially superimposed upon the line obtained above by serial regressions. The predicted LC_{10} and LC_{90} lines enclose the majority of the data

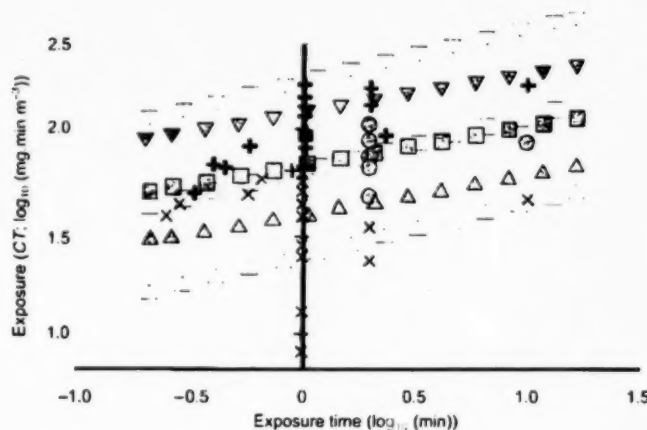


Figure 5. Comparison of dog and pseudo-dog calculations. The accepted dog data are plotted showing 0–20% (X), 20–80% (●) and 80–100% (+) toxic results. Each symbol represents a group (1 to 4 animals) tested at that CT. The calculated real dog LCt₀₅ (▲), LCt₅₀ (■) and LCt₉₅ (▼) lines and the calculated pseudo-dog LCt₀₅ (○), LCt₅₀ (□) and LCt₉₅ (◇) lines are superimposed. Both the dog and pseudo-dog 2V surface models would adequately represent the real dog data

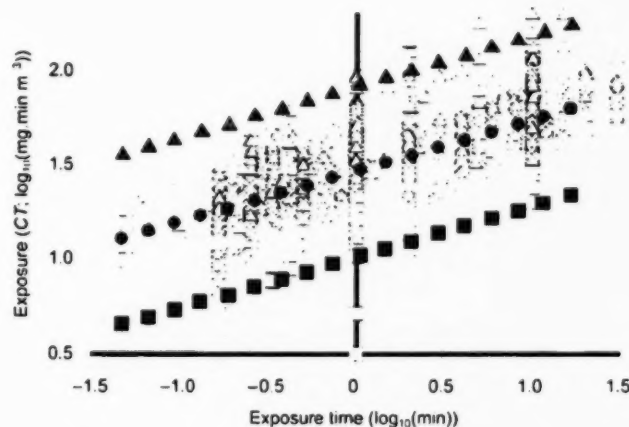


Figure 6. The pseudo-human data distribution shown with LCt₀₅, LCt₅₀ and LCt₉₅ lines from the calculated surface. The pseudo-human data are plotted to show 0–20% (○), 20–80% (◐) and 80–100% (◑) toxic responses. Each symbol represents the CT value of one 'experimental' group. The LCt₀₅ (■) and LCt₉₅ (▲) lines include all but a few of the data points and those outside points represent either low or high responses. The LCt₅₀ line (●) effectively bisects the distribution

set and the lines appear closely predictive of the pseudo-human distribution suggesting that reasonable confidence may be placed in the toxicity values indicated (Table 11).

Close inspection of the pseudo-human data set (Fig. 6) indicated that the distribution of values might be compacting as the exposure time shifted from low seconds to minutes, i.e. the distribution appeared to compress as the exposure time increased. To test this concept, several 2V surfaces were calculated from the pseudo-human data set, progressively removing the data for shorter exposure times (Table 7). As the lower exposure limit increased in

four steps from 0.17–0.99 min, the *n* values of the four calculated surfaces remained essentially constant (mean $\pm$ standard deviation: 1.399 ± 0.011), the slope constants *b*₁ and *b*₂ increased, the Ratio decreased by 33% and the predicted LCt₀₅ values (Table 10) remained constant. These changes indicated that the distribution was compacting without changing the central axis of the predicted 2V surface. A further change in the lower limit to 1.99 min produced changes in the Ratio and slope constants that would have been considered as improvements. However, there was a sharp increase in the predicted

Table 10. Toxicity estimates for 70 kg humans breathing 15.6 l min⁻¹

Item	Exposure time (min)	Toxicity (mg.min m ⁻³)			
		LCt ₀₁	LCt ₀₅	LCt ₅₀	LCt ₉₅
2 min exposure					
[1]	0.1 ^a 30	8.1	12.4	35.2	100
[2]	0.33 30	10.1	14.6	35.0	84
[3]	0.5 30	9.9	14.3	34.6	83
[4]	1 30	10.6	15.0	34.7	80
[5]	2 30	11.4	15.8	39.7	77
10 min exposure					
[6]	0.17 30	12.6	19.4	55.2	157
[7]	0.33 30	16.0	22.9	55.1	132
[8]	0.5 30	15.9	22.9	55.3	133
[9]	1 30	16.9	23.9	55.1	127
[10]	2 30	17.9	24.9	62.6	122
30 min exposure					
[11]	0.17 30	17.2	26.4	75.1	213
[12]	0.33 30	21.7	38.0	75.0	180
[13]	0.5 30	21.9	31.6	76.2	184
[14]	1 30	23.2	32.8	75.6	175
[15]	2 30	24.5	34.0	85.4	166
Mean ± SD	Items [1–4]		34.9 ± 0.27		
	Items [6–9]		55.2 ± 0.10		
	Items [11–14]		75.5 ± 0.45		

Table 11. Recommended toxicity estimates for a 70 kg human

Minute volume (l min ⁻¹)	Exposure time (min)	Toxicity (mg.min m ⁻³)			
		LCt ₀₁	LCt ₀₅	LCt ₅₀	LCt ₉₅
6	2	27	40	65	207
	10	45	62	142	330
	30	60	85	197	455
15	2	11	16	36	83
	10	18	25	57	132
	30	24	34	79	182
30	2	5.5	8.0	18	42
	10	9.0	12	28	66
	30	12	17	40	91

LCt₀₁ (Table 10) of more than 3 standard deviations compared with the mean of the previous values. Therefore, the 2V surface calculated for the range 2–30 min was not considered further.

Recommended Toxicity Values

The procedures above provide a pseudo-human database that is derived directly from the animal data and a 2V planar model of the data from which specific toxicity estimates may be obtained. There was no arbitrary selection of data as all data used met the applied inter-laboratory quality assurance criteria applied. The 2V planar model fits the data distribution (Fig. 6; Tables 7, 10). The human LCt₅₀ vs *T* estimates are generally consistent and representative as the data set is manipulated

(Table 10). As demonstrated by serial calculations of the model with an increasing lower limit (Table 7), there is a much greater variability in the predicted mortality data at the shorter exposure times. The difficulty in generating exposures for the short times may be part or all of the cause of the increased variability at the low exposure times. Biologically, an exposure 10 s to a human may be academic. Even if the human inhales during the exposure period, the short *T* would be meaningless as the time for inhalation would exceed the *T* and be the more correct exposure time. Further, at a breathing rate of 11–17 breaths per min or 5.5–3.5 s per breath (Altman and Dittmer, 1956; Snyder *et al.*, 1975; Myles and Sanders, 1979), a wide variation can occur in the amount of contaminated air inhaled in a 10 s exposure according to where the subject is in the breathing cycle as the

exposure is started and, if the source of vapour is relatively close to the subject, the variability of concentration in the chemical cloud (Yee and Ye, 1996; Yee, 1999) will add another large uncertainty to the delivered exposure. Therefore, despite the wide distribution of toxic effects indicated, the model for 0.17–30 min exposures may be the choice when very short exposures are considered with the subjects close to the source. However, under more general conditions where the exposure times are longer so the breathing cycle has less effect and under conditions where the source is more distant so that the concentrations within the cloud are less variable, the steeper planar model for the 1–30 min exposure times would appear more suitable. As the former conditions may be a special case, the recommended toxicity estimates for general use would be those generated from the model calculated for the 1–30 min exposures.

The recommended toxicity estimates for a 70 kg human breathing 6, 15 and 30 l min⁻¹ are given in Table 11. Direct calculations from the 2V surface equation for 1–30 min exposures provide toxicity values for a 15.6 l min⁻¹ minute volume (Table 10). The 15.6 l min⁻¹ minute volume for the human is the value obtained for a 70 kg subject from the allometric relationship of minute volume to body weight for many species (Bide *et al.*, 1997, 2000). The toxicity values for 6, 15 and 30 l min⁻¹ were obtained by multiplying by the ratios of the minute volumes involved. Values for other minute volumes may be obtained in a similar fashion. However, a simple ratio calculation should not be used with other human body weights. A calculation from the allometric relationship between minute volume and body weight should be used instead.

Discussion

A procedure is presented that provides an estimation of human toxicity that is totally dependent upon direct calculations from the animal data. Deliberately, there are no 'selections' of (critical) data or experiments, no inferences of values, no assumptions based upon other data and no subjective assessments of studies to include or reject the data. The procedure described creates a 'pseudo-human' data set from the animal toxicity data and then fits a mathematical, planar, 2V surface model to that data set. The 2V surface model predicts that the toxicity parameters, notably b_1 (the 1V Normit slope at T) and b_2 , are constant as the exposure time changes. A surface calculated using all of the pseudo-human data created from the accepted animal data, appears to represent the total data set for GB toxicity very well as almost all of the critical 20%–80% mortality data fall between the LC_{50} and LC_{95} lines (Fig. 6). The key to the process is to achieve the situation described in Fig. 3. The relationship between toxicity and exposure time may be different

from the one presented in this paper but the process presented will still work effectively as long as the toxicity-exposure time relationships produce near parallel lines for each species as shown in Fig. 3. Once this point has been achieved, the mathematics and procedures required to produce the pseudo-human data set, the predicted human LC_{50} lines and the human estimates are trivial.

Comparisons of physiological function between the species may be done by several allometric methods relating function to body size, weight, surface area, etc (Phalen, 1984). For respiration, the historical comparisons have been minute volume compared with body weight (Guyton, 1947; Crofford and Widdicomb, 1961; Snyder *et al.*, 1975; Blackburn, 1984; Phalen, 1984; Snipes, 1988; West *et al.*, 1997) usually as the minute volume/body weight ratio. However, in the listed papers, there was no separation between anaesthetized and non-anaesthetized animals. In previous papers from this laboratory (Bide *et al.*, 1997, 2000), an allometric relationship between minute volume and body weight was developed for young adult, non-anaesthetized mammals. Using this relationship the equivalent minute volume for a 70 kg human and the equivalent MV/BW were determined to be 15.6 l min⁻¹ and 0.223, respectively. These values were used as the human respiratory parameters in the present study.

The toxicity values (LC_{50} values generated from the 2V human surface are in good agreement with literature values for the LC_{50} of GB. The most recent estimation of human toxicity for GB, provided (Koller and Henderson, 1997; Grotte and Yang, 1998) for the U.S. Army as part of the preparations for the Gulf War (Operation Desert Storm) was 35 mg.min m⁻³ for a 2 min exposure to a 70 kg human breathing 15 l min⁻¹. (This breathing rate, considered as a 'standard' for military purposes, represents a fit man doing light work (Snyder *et al.*, 1975; Myles and Sanders, 1979). The 2 min value from the present study is 36 mg.min m⁻³ (Table 11). Earlier human toxicity estimates from the literature (Wills and DeArmon, 1954; Christensen *et al.*, 1958; Cresthull *et al.*, 1961; Anon., 1967, 1970; Somani *et al.*, 1992; Harber, 1993; Marrs *et al.*, 1996; Sidell, 1998; Russian Munitions Agency, 2002) indicate (assumed) 10 min LC_{50} values of 50–100 mg.min m⁻³ (several of these are based upon or refer back to the same original papers for the numbers), values closer to the 10 min toxicity value of 57 mg.min m⁻³ calculated by the procedures described in this report. All these early LC_{50} estimates are in essential agreement given the variations in methods, exposures, breathing rates and other available information. None of the previous attempts to estimate human toxicity provided either a continuous surface describing the exposure time-concentration-toxicity response or human LC_{50} and LC_{95} values generated directly from the animal data.

The Normit (PROBIT) slope of 4.5 predicted by the 2V model, however, is much less than the estimates from

many individual animal studies. Values between 7 and 23 have been reported (Cresthull *et al.*, 1957, 1961; Christensen *et al.*, 1958; Koller and Henderson, 1997; Grotte and Yang, 1998; Mioduszewski *et al.*, 2000, 2001; Bide and Risk, 2002, 2004;). The Normit value, in turn, affects the predicted LC_{50} values. Opinion varies among authors as to the best way to predict the slope (Normit, b_1 , b_2) values for the human population. In one concept, both the LC_{50} and Normit slope are to be derived from 'selected' animal experimental data representing the most sensitive animal species (Fairhurst and Turner, 1993; Franks *et al.*, 1996). In this process, derived for public risk assessment from meagre animal toxicity data, the selected Normit slope is as steep as is found in laboratory animal toxicity testing. Use of the process for scientific purposes is questionable. Although the differences between carefully selected, closed animal populations and the widely variable human population are acknowledged (Fairhurst and Turner, 1993), involving the increased variability of the human population is discounted and ignored as unnecessary. The concept seems to be that the variations seen in animal toxicity values are experimental (particularly inter-laboratory) rather than biological. This view ignores even the simple differences in toxicity that will arise from the acknowledged body weight range of about 150 to 300 lb within a healthy male, 25 year old, 6 foot human population. The opposing view is followed in this study — that all attempts to obtain human estimates from animal data must account for the difference between the genetically similar populations of the test animals, reared in isolation, deliberately selected to be the same sex, near constant weight and age, guaranteed not to have x , y , z respiratory diseases, fed on a constant diet and the target human population that is genetically diverse, disease carrying, nutritionally very diverse, and of mixed age, sex, weight and body style (Dixon, 1976; Krasovskii, 1976; Blackburn, 1984; Anon., 1994, 1998). As expected, toxicity testing of the 'outbred' laboratory animal strains produces tight, steep exposure response data (Cresthull *et al.*, 1957; Mioduszewski, 2000, 2001; Bide and Risk, 2002) albeit that a wide variation in Normit slopes can be encountered here also (in monkeys, 3.3–17.6; Cresthull *et al.*, 1957). When randomly selected, genetically diverse subjects such as the population of dogs and monkeys (Fig. 5; Tables 5–7) are used, the Normit slopes are lower and the data show greater variability. Indeed, even serial results in the same laboratory with supposedly similar animals from the same source, produce increased variability (McPhail, 1955; Cresthull *et al.*, 1957; Mioduszewski *et al.*, 2000, 2001; Bide and Risk, 2002, 2004). In the current study, inter-laboratory variability is recognized and a process is described to provide a modicum of inter-laboratory quality control in the data base. No claim is made that the remaining variability is totally biological but rather that the biological variability

in the toxicity estimates is better represented by the observed combination of experimental and biological variation than by artificial 'selection' and combination of experimental data that ignores the biological variability of both experimental animals and humanity. The author's opinion is that the 4.5 Normit value from the pseudo-human surface better reflects the human population: — adult men and women of all sizes, ages, race, ethnicity and physiology/physiognomy.

Notwithstanding the above, when data for the exposure times ≤ 0.99 min were progressively removed from the surface calculations, the predicted LC_{50} values were very similar (Table 11) but b_1 , b_2 and the Ratio clearly indicated that the predicted surface was becoming steeper. In turn, this indicates that the variability seen in the very short exposure times was greater. As noted, this greater variability at very short exposure times may result either from technical considerations in generating the exposures or from biological variations in the breathing rates and timing of the exposures. However, the result may be fortuitous in view of the large concentration differences seen in a chemical cloud (Yee, 1996a; Yee and Ye, 1996) and the resulting variability of the exposure to individuals separated by only short distances. Because the toxicity is not directly related to exposure, the values used for a given project/purpose should be chosen to best fit the scenario in question. The recommended values for general use are those calculated from the 1–30 min exposure range (Table 11). When very high transient exposures are involved, as when the subjects are close to the source of a cloud, the values for the 0.17 to 30 min toxic load model may be more appropriate.

A cautionary caveat must be clearly understood. The analysis and estimates presented are based upon empirical relationships. As such, the range of exposures for which human toxicity estimates may be made is limited by the range of the animal toxicity data available. Mathematical extrapolation beyond the boundaries of the original animal data is possible (the calculated lines are mathematically limitless) but this is not a recommended practice and may well lead to serious error. When this project began, there were only few acute toxicity data available in the literature for exposure times longer than 15 min and these were only for the rat (Rotariu *et al.*, 1945). From current work, which extends the rat (Mioduszewski *et al.*, 2000, 2001) and mouse (Bide and Risk, 2002, 2004) data to longer times, it is clear that the empirical C^*T or toxic load relationship described in this study may be invalid for exposure times of >30 min. Similarly, toxicity at lower exposure times may be estimated but in this case, the surface calculated for 0.17 to 30 min, which predicts the same LC_{50} , may provide better toxicity estimates for LC_{50} .

The concepts and the analytical methods presented here cannot be validated by comparisons to human toxicity data. Therefore, to demonstrate the functionality

of the procedures proposed, a 'pseudo-dog' data set was prepared in the same method as proposed for the human (Tables 5, 7). The C^*T lines for the actual dog and the calculated 'pseudo-dog' data are close and cross. However, from Fig. 5 it is clear that either empirical line can effectively represent the dog data.

This report describes a part of an ongoing study. The human inhalation toxicity estimates given in this report are constantly being refined and updated as more information becomes available. In particular, the data for the rat and mouse are under close scrutiny at this time in an attempt to reduce the dispersion of these data. However, the amount of data already available for calculating the human estimates is such that the addition of more data within 10 s to 30 min exposure times should have only minor effects upon the outcome of the calculations. Similarly, the effects on the statistical calculations of small numbers of aberrant data points would be reduced by the weight of numbers.

As in any calculation of this type, the toxicity estimates are only as good as the original data available. To minimize the effect of inter-laboratory differences, the procedure described includes a critical comparison of the data from each study to the total data for each species. In the example used, GB, there are many studies from different laboratories so that systematic errors in any one location should be either eliminated because the data would be different from the remaining studies on that species or, if of small magnitude, swamped by weight of numbers. However, nothing will protect against a systematic or procedural error across all studies. In the inter-laboratory quality control process used, the data from 11 studies were discarded. In one (McPhail, 1955), all the data were for exposure times of <10 s. In another (Koon *et al.*, 1960), the procedures described strongly suggest that the high values obtained may have been caused by rebreathing of the test atmosphere (Moss and Asgharian, 1994; Wong, 1999). With the exception of the one study noted (Koon *et al.*, 1958), there was nothing in the reports to indicate a reason/cause for the discarded studies to digress from the remainder. In the animal data, there is a preponderance of data from mice and rats and, unfortunately, it is these data that, initially, have the greatest variability. In the case of mice, a strain susceptibility to organophosphates has been reported (Clement *et al.*, 1981). Despite this, the animal studies and the predicted human values appear to be consistent within themselves and, in the authors' opinion, represent the best currently available estimates of the inhalation toxicity of GB for a large human population.

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DRDC Suffield Animal Care Statement

In conducting the research described in this report, the investigators adhered to the *Guide to the Care and Use of Experimental Animals*, Vol. I, 2nd edn published by the Canadian Council on Animal Care.

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